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Effects of Dietary Fat, Vitamin E and Zinc on Immune Response and Blood Parameters of Broiler Reared Under Heat Stress

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Abstract: The purpose of this study was to determine the effect of corn-soybean basal diets containing (5% tallow, 5% fish oil and 5% fish oil with vitamin E and zinc) each one in two temperatures (21 and 32°C) on immune response and blood parameters of Arbor Acres (AA) broiler growing from 1-42 days of age. The results demonstrated that heat stress decreased bursa of fabricius weight and spleen weight also high ambient temperature decreased humoral and cell-mediate immune responses and increased hetrophil to lymphocyte ratio ($H L^{-1}$). Fish oil+vitamin E and zinc diet improved humoral and cell-mediate immune responses, decreased $H L^{-1}$ ratio and increased bursa of fabricius and spleen weight at two temperatures. The diets containing tallow decreased bursa of fabricius weight and spleen weight. In chicks fed by fish oil, the response to 2-4 Dinitrochlorobenzene (DNCB) was improved. It did not have significant influence on Phytohemagglutinin M (PHA-M) and Sheep Red Blood Cells (SRBC). The results of experimental temperatures on blood parameters demonstrated that high ambient temperature increased cholesterol, triglyceride, glucose, hematocrite percentage and serum Malondialdehyde (MDA), although it did not affect total protein. Higher cholesterol and triglyceride observed in chicks fed with diet containing tallow and higher MDA in chicks fed with diet containing fish oil. Fish oil plus vitamin E and zinc decreased cholesterol, triglyceride, MDA and glucose significantly. Fat type did not affect total serum protein and hematocrite percentage.

Key words: Broiler, heat stress, fatty acid, blood parameters, immunity, spleen

INTRODUCTION

Environmental temperature influence poultry immune response. Heat stress causes suppressive result on immune system. High ambient temperature during rearing is associated with an increase in the stress status of broilers which is measured by hetrophil to lymphocyte ratio (Yalcin *et al*., 2003). The several systems that participate in thermoregulation operate through modulation of heat production and/or heat loss. The cardiovascular system may affect both processes on one hand, modulation of heat dissipation and by oxygen transport on the other. Plasma volume expansion was observed during the need for acute heat dissipation such as a period of heat exposure in rats (Meiri *et al*., 1991). It is not clear whether changes in the blood system are part of acclimation to high or low environmental temperatures or are the response to acute perturbation only. Unsaturated fatty acids stimulate the body's physiological processes during stress (Cook *et al*., 1993; Miller *et al*., 1994). Whitehead (2000) holds that by stimulating the immune system through modified feeding, it is possible to offset the negative effects of reduced immunity resulting from stress. Several methods are available to alleviate the negative effects of high environmental temperature on performance of poultry. Because of the high cost and impracticality of cooling animal buildings, interest in dietary manipulations has increased. Studies have shown that antioxidant nutrient supplementation especially vitamins C, E and A, zinc and chromium can be used to attenuate the negative effects of environmental stress (Kafri and Cherry, 1984; Mowat, 1994). Because α-tocopherol protects PUFA from lipid oxidation, its inclusion in the bird's diet may result in a higher deposition of PUFA in poultry tissues.

Supplemental zinc used in poultry diets is beneficial to layer hens during environmental stress (Sahin and Kucuk, 2003). Dardenne *et al*. (1985) demonstrated that zinc increase cell immune purification and increase produced antibody. Environmental stress has been shown to decrease serum and tissue levels of antioxidant vitamin in poultry (Sahin and Kucuk, 2003). As a primary

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antioxidant of cell temperance, vitamin E is particularly important for the prevention of fatty acid peroxidation (Benedich, 1990). Interactions among minerals and other nutrients e.g., vitamin E are extensive and may be important in the determination of biological availability of other nutrients. The reduced vitamin E, A, zinc and copper caused reduced immune response (Beisel, 1982). Fatty acid can act as immunoregulatory molecules that mediate cellular communication, membrane fluidity and second messenger elaboration (Klasing, 1997) and the effect of Vitamin E on fatty acid stability may be immunoregulatory in itself.

Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds in blood and tissues. One of the products is Malondialdehyde (MDA) which has long been considered as an index of oxidative rancidity. Among all the methods proposed for assessing MDA, the 2-Thiobarbituric Acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. Environmental stress causes oxidative stress and impairs antioxidant status *in vivo* (Halliwell and Gutteridge, 1989; Sahin *et al*., 2001; Splettstoesser and Schuff-Werner, 2002). The primary source of Reactive Oxygen Species (ROS) is leakage of electrons from the respiratory chain during the reduction of molecular oxygen to water to generate superoxide anion. In chickens heat stress causes metabolic changes in substrate oxidation and ROS production in mitochondria.

The objective of this study is to evaluate effects of fat type and antioxidant on immune response and blood parameters in broilers reared under heat stress.

MATERIALS AND METHODS

About 240 female Arbor Acres broiler chicks were utilized in a 6 week experiment designed to assess the effects of diet and temperature on the immune response and blood parameters. This experiment has done in Tarbiat modarres university of Iran. The experimental treatments and diets were identical to those utilized in experiment. The chicks were placed in floor pens equipped with stainless steel feeders and automatic water drinkers. Effect of diet and temperature were studied using a 2×3 (temp × feed) factorial design of treatments: 6 treatments with 4 replications and 10 birds in each one. The temperatures was maintained at approximately 21°C (normal) and 32°C (heat stress) using thermostatically controlled heaters and exhaust fans in two chambers. Lighting was continuous and water and feed were available *ad labium* for 42 days. The diets includes: basal diet with 5% Tallow, basal diet with 5% fish oil, basal diet with 5% fish oil plus vitamin E and zinc (100 IU vitamin E and 50 mg kg^{-1} zinc) (Table 1). In order to evaluate the immunosuppressive effect three chicks from each replication (generally twelve chicks from each treatment) were used. In 35 days old, 0.2 μL sof SRBC 5% was injected into breast muscle of the broilers. After 5 and 9 days post injection, 4 mL of blood was drawn from the brachial vein using heparinized syringes. Whole blood was used to prepare smears for H/L (heterophil to lymphocyte). Antibody production in response to SRBC (Sheep Red Blood Cell) was measured at 5 and 10 days post inoculation by the micro titer haemagglutination method.

Delayed-type hypersensitivity a measure of cell mediated immunity was evaluated by the phytohemagglutinin-Minduced wattle swelling assay as described by Klasing (1998). Briefly, chicks were injected on day 42 with 100 μL of PHA-M (0.1 mg mL^{-1}) into the right wattle only phosphate buffered saline was injected into the left wattle. About 12 and 24 later, the thickness of each wattle was measured using a micrometer. The ratio of the thickness of the PHA injected wattle to the thickness of the PBS-injected wattle is the wattle index. Measurement of cell-mediate immune responses to PHA-M (phytohemagglutinin M) carried out as explained by Li *et al*. (1999) and DNCB (2-4 dinitrochlorobenzene) stimulate cell mediated immunity used Thompson *et al*. (1980) method. To consider the effect of treatments on blood parameters, three chicks from each replication (generally twelve chicks from each treatment) were used. About 2 mL of blood was drawn from the brachial vein blood samples and centrifuged at 3,000 g for 10 min therefore, sera were collected. Blood characteristics included in the study were whole blood hematocrit, serum protein, serum cholesterol, serum glucose, serum triglyceride and serum malondialdehyde. Hematocri expressed as blood percentage of packed cell (primarily red blood cell) volume was determined through use of capillary tubes that were centrifuged in a micro-HCT centrifuge and were then read with a micro-capillary reader. Serum cholesterol, Serum triglyceride, serum protein, serum glucose were determined using enzymatic methods using diagnostic kit (Pars Azmon). Serum malondialdehyde was determined by the method of Chang *et al*. (1998).

Data were analyzed separately, using the GLM procedures of SAS software package (SAS institute, 1999). Treatment means for all measurements were separated by Duncan means procedure at significant level of $p>0.05$.

Table 1: Ingredients and chemical analyses of the starter and grower diets fed to broilers reared under heat stress

	Treatments					
	Starter percentage of DM			Grower percentage of DM		
Ingredients	O	A	T	O	A	T
Ground corn	54.75	54.75	54.00	61.73	61.73	61.00
Soybean meal	37.28	37.28	37.28	30.25	30.25	30.25
Fish oil	4.51	4.51	-	4.65	4.65	-
Tallow	-	-	5.26	-	-	5.38
Dicalcium phosphate	1.37	1.37	1.37	1.33	1.33	1.33
Sodium chloride	0.10	0.10	0.10	0.20	0.20	0. 2
Limestone ground	0.53	0.53	0.53	0.53	0.53	0.53
Calcium carbonate	0.10	0.10	0.10	0.16	0.16	0.16
DL-methionine	0.24	0.24	0.24	0.25	0.25	0.25
Lysine	-	-	-	0.12	0.12	0.12
Cavilamycin	0.10	0.10	0.10	0.10	0.10	0.10
Ca-propionate	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin E IU	-	100.00	-	-	100.00	-
Zinc mg Kg^{-1}	-	50.00	-	-	50.00	-
Clinaox	0.10	0.10	0.10	0.10	0.10	0.10
Toxinil	0.30	0.30	0.30	-	-	-
Vitamin premix*	0.27	0.27	0.27	0.27	0.27	0.27
Trace mineral premix**	0.16	0.16	0.16	0.16	0.16	0.16
DM (%)	89.65	89.65	89.63	89.68	89.68	89.68
Chemical analyses						
ME, kcal kg^{-1}	3100.00	3100.00	3100.00	3200.00	3200.00	3200.00
Cp	23.00	23.00	23.00	20.03	20.03	20.03
Crude fat	7.04	7.04	7.04	6.35	6.35	6.35

*Premix supplied for 2 kg: vitamin A, 15000 IU; cholecalciferol, 3 IU, vitamin E 15 IU; menadione, 2.5 mg; vitamin B1, 1 mg vitamin B2, 10 mg; niacin, 70 mg; d-pantotheenic acid, 20 mg; vitamin B12, 4 mg; folic acid, 2 mg; biotin, 0.1 mg; **premix supplied: 40 mg Mn kg^{-1}, 12.5 mg Fe kg^{-1}, 25 mg Zn kg^{-1}, 3.5 mg Cu kg^{-1}, 0.15 mg Iodine kg^{-1}, 0.075 mg Se kg^{-1}, 175 mg Choline chloride kg^{-1}

RESULTS AND DISCUSSION

There was no significant difference in feed to gain ratio of broiler chickens between dietary treatments but high environmental temperature reduced the feed intake, body weight and increased feed to gain ratio in broiler. treatments on performance parameters are shown in Table 2. This result showed that tallow diet had significant effect on feed intake and body weight, although the same result on feed to gain was not convenient ($p>0.05$).

It was may be due to high energy level of tallow in comparison with fish oil. At the other hand addition of omega 3 fatty acid to the diet reduces fat content of blood and body so this leads to reduce body weight of broilers. Addition of zinc and vitamin E to the fish oil ration caused increasing in body weight of broilers though it was less than body weight of Tallow ration. Introduction of zinc and Vitamin E to fish oil diet reduces MDA production and this may ameliorate the negative effects of oxidative peroxidation and heat stress on body weight of broiler chickens. Interaction of diet and temperature significantly affects broilers performance ($p>0.05$). As shown in Table 1, High environmental temperature with fish oil diet had the worst effect on Feed to gain ratio, feed intake and body weight of broiler chickens. High ambient temperature and high polyunsaturated diets increase susceptibility to peroxidation of lipids in plasma and body tissues so this causes reducing in performance of broilers. Negative effect of heat stress on growth rate and production is speculated to be due primarily to reduced feed intake (Hurwitz *et al*., 1980). The effects of dietary fat and temperature on immune response are shown in Table 4. High environmental temperature was significantly decreased the DNCB, PHA-M index after 12 and 24 h, primary and secondary antibody production against SRBC, spleen and bursa weight but H/R ratio increased by heat stress ($p>0.05$).

High ambient temperature reduced body response to PHA-M since it may stimulate corticostron production which has negative effect on epidermal basophiles stimulated by PHA-M. This data shown that heat stress significantly affect the immune response and immunity level of broiler chickens. The addition of Vitamin E and zinc to fish oil diet significantly increase T lymphocytes, phagcytosis, SRBC antibody titer and weight of spleen and bursa (percentage of body weight) but reduced the H/T ratio. These results indicated that addition of antioxidant ameliorated the negative effects of heat or high polyunsaturated diets on immune response of broiler

Table 2: The effect of temperature and diet on feed intake, feed to gain and body weight

Parameters	Feed intake	Feed to gain ratio	Body weight
Temperature			
High (H)	4976^{B}	1.96^{A}	2539^{B}
Normal (C)	6329^{A}	1.89^{B}	3349^{A}
SEM	-	-	-
Diet			
T	5929^{A}	1.92	3088^{A}
O	5423^{B}	1.93	2810^{B}
A	5662^{AB}	1.93	2934^{AB}
SEM	-	-	-
Interaction (Temp x diet)			
TH	5297^{C}	1.95^{A}	2717^{B}
TC	6539^{A}	1.89^{B}	3460^{A}
OH	4682^{D}	1.97^{A}	2377^{C}
OC	6131^{B}	1.89^{B}	3244^{A}
AH	4947^{CD}	1.96^{A}	2524^{BC}
AC	6301^{AB}	1.89^{B}	3334^{A}
SEM	192	0.23	88
Main effect (p-value)			
Temperature	*	*	*
Diet	*	NS	*
Temperature x diet	*	*	*

$^{A, B}$column means with common superscripts do not differ *p>0.05; **p>0.01; NS-None Significant, O: Fish Oil, A: O diet+100 IU vitamin E kg^{-1} and 50 mg zinc kg^{-1} T: Tallow, OH: High temperature and O diet, OC: Normal temperature and O diet, AH: High temperature and A diet, AC: Normal temperature and A diet. TH: High temperature and T diet TC: Normal temperature and T diet

Table 3: The effect of temperature and diet on immune response of broiler

			PHA-M		SRBC			
Parameters	DNCB 12 h	H/L	12 h	24 h	Primary	Secondary	Spleen	Bursa
Temperature								
High (H)	0.48^{B}	0.40^{A}	0.40^{B}	0.54^{B}	1.66^{B}	3^{B}	0.065^{B}	0.045^{B}
Normal (c)	0.86^{A}	0.25^{B}	0.52^{A}	0.78^{A}	2.58^{A}	5.16^{A}	0.083^{A}	0.053^{A}
SEM								
Diet								
T	0.64^{B}	0.35^{A}	0.43	0.65	1.62^{B}	3.31^{B}	0.0756^{AB}	0.049^{AB}
O	0.77^{A}	0.36^{A}	0.45	0.68	2.06^{AB}	3.12^{B}	0.0602^{B}	0.044^{B}
A	0.58^{B}	0.27^{B}	0.5	0.66	2.68^{A}	5.81^{A}	0.087^{A}	0.054^{A}
SEM								
Interaction (Temp x diet)								
TH	0.41^{D}	0.43^{D}	0.38^{C}	0.54^{C}	1.13^{C}	2.13^{C}	0.086^{AB}	0.047^{B}
TC	0.87^{A}	0.28^{D}	0.54^{A}	0.76^{B}	2.13^{B}	4.50^{B}	0.055^{C}	0.053^{A}
OH	0.65^{C}	0.47^{A}	0.34^{D}	0.55^{C}	1.63^{C}	2.25^{D}	0.066^{BC}	0.041^{B}
OC	0.89^{A}	0.26^{D}	0.53^{AB}	0.81^{A}	2.50^{B}	4.60^{B}	0.076^{B}	0.047^{AB}
AH	0.35^{E}	0.33^{E}	0.51^{AB}	0.56^{C}	2.25^{B}	4^{B}	0.099^{A}	0.049^{A}
AC	0.82^{B}	0.23^{D}	0.49^{B}	0.81^{A}	3.13^{A}	7^{A}	0.099^{A}	0.06^{A}
SEM	0.045	0.019	0.017	-	0.145	0.34	0.003	0.0013
Main effect (p-value)								
Temperature	*	*	*	*	*	*	**	**
Diet	*	*	NS	NS	*	*	*	*
Temperature x diet	*	*	*	*	*	*	*	*

$^{A, B}$column means with common superscripts do not differ *p>0.05; **p>0.01; NS-None significant, O: fish oil, A: O die +100 IU vitamin E kg^{-1} and 50 mg zinc kg^{-1} T: Tallow, OH: High temperature and O diet, OC: Normal temperature and O diet, AH: High temperature and A diet, AC: Normal temperature and A diet. TH: High temperature and T diet TC: Normal temperature and T diet

chickens. Vitamin E causes H/L ratio increased, indicating that it improved the phagocytic capacity of the immune system, protecting the birds against the invasion of pathogenic microorganisms (Boa-Amponsem *et al*., 2000). Antibody titer in rations containing Poly Unsaturated Fatty Acids (PUFA) was higher than tallow ration since it was probably due to positive effect of omega3 PUFA and negative effect of omega6 PUFA on humoral and cell mediated immune system (Fritsche *et al*., 1991). Environmental stress has been shown to decrease serum and tissue levels of antioxidant vitamin (Sahin and Kucuk, 2003) and increase copper and zinc extraction. Belay and Teeter (1996) declared that these agents decreased immune response. Psychological and physical stressors such as fasting, frustration, water deprivation, crowding and heat stress increase the ratio of Heterophil to Lymphocyte (H/L) (Jones, 1989; Cravener *et al*., 1992). Fat type had influence on immune response. Diet

Table 4: The effect of temperature and diet on blood parameter of broiler reared under heat stress

Parameters	Cholesterol (mg dL^{-1})	Triglycerid (mg dL^{-})^{1}	Glucose (mg dL^{-})^{1}	Protein (mg dL^{-1})	Serum MDA (nmol dL^{-1})	Hematocrit (%)
Temperature						
High (H)	133^{A}	63.3^{A}	0.4^{A}	2.5	1.04^{A}	27.75^{A}
Normal (c)	101^{B}	46.57^{B}	0.25^{B}	2.5	0.80^{B}	25.25^{B}
SEM	-	-	-	-	-	-
Diet						
T	123.2^{A}	67.5^{A}	190^{B}	2.4	0.88^{B}	26.6
O	117.2^{B}	52^{B}	234^{B}	2.7	1.17^{A}	26.7
A	103.3^{C}	45.4^{C}	199^{C}	2.3	0.75^{C}	26.12
SEM	-	-	-	-	-	-
Interaction (Temp x diet)						
TH	145.8^{A}	73.6^{A}	192.6^{D}	2.99^{A}	0.849^{C}	28.2^{A}
TC	118.5^{C}	61.2^{B}	187.7^{D}	2.27^{B}	0.64^{D}	25^{C}
OH	137.8^{B}	62^{B}	247.9^{A}	2.93^{A}	1.18^{A}	27.7^{A}
OC	96.7^{D}	42.08^{C}	220.1^{B}	2.12^{B}	1^{B}	25.7^{BC}
AH	116.7^{C}	54.5^{D}	202.3^{C}	2.95^{A}	0.96^{B}	27.25^{AB}
AC	89.9^{E}	36.38^{A}	196.3^{CD}	2.1^{B}	0.753^{C}	25^{C}
SEM	2.2	2.64	1.44	0.07	0.01	0.32
p-value	0.042	0.035	0.021	0.048	0.041	0.029
Main effect (p-value)						
Temperature	*	*	*		*	*
Diet	*	*	*		*	
Temperature x diet	*	*	*	*	*	*

$^{A, B}$column means with common superscripts do not differ *p >0.05; **p >0.01; NS-None Significant, O: fish oil, A: O diet+100 IU vitamin E kg^{-1} and 50 mg zinc kg^{-1} T: Tallow, OH: High temperature and O diet, OC: Normal temperature and O diet, AH: High tem-perature and A diet, AC: Normal temperature and A diet. TH: High temperature and T diet TC: Normal temperature and T diet

supplementation by fish oil improved response to DNCB but did not influence on PHA-M and SRBC responses. Phytohemaglutinine stimulates cell mediated immunity in broilers. Diets did not have significant effect on PHA-M challenge even though the interaction of diet and temperature significantly affected PHA response so that the highest and lowest response after 12 h belonged to TC and OH treatments, respectively. These results are inagreement with thoese of Selvaraj and Cherian (2004).

These results are in agreement with the results of Wang *et al.* (2000). The highest secondary response to PHA-M belonged to AC treatment which probably characterized as the effects of antioxidant and zinc on cell mediated immunity. Diet enriched with USFA (n-3) has anti-inflammatory properties (Korver and Klasing, 1997) increased antibody responses and decrease lymphocyte proliferation (Fritsche, 1991). Enhanced serum antibody production was also observed by Friedman and Sklan (1995) in broiler chicken fed a high n-3 PUFA diet. Higher immune response for DNCB and PHA-M was found in TC treatment and higher antibody titer was evidenced in AC treatment. Also higher H/L ratio was found in OH treatment. Cell-mediated immunity as indicated by cutaneous basophile hypersensitivity to PHA-P (measured by determining wattle index) was suppressed to approximately the same degree as reported by single depot injections of ACTH in broilers (Murray *et al*., 1987; Post *et al*., 2003). Fat type and fatty acid composition of diet (especially omega3 fatty acids) significantly affect the immunity response of broiler chickens. Rearing temperature had significant effect on spleen and bursa of fabricius weight (percentage of body weight) ($p>0.05$). Heat stress decreased spleen and bursa of Fabricius weight but there was not significant increase for fat type. Supplemental vitamin E and zinc resulted in a significant improvement of spleen and bursa of Fabricius in heat stress broiler chicks. Affected hematocrit values and Hematocrit values vary with the ambient temperature in which birds are reared. The effects of diet and temperature on blood parameters are shown in Table 4. The results showed that heat stress increased hematocrit percentage, Serum MDA, serum cholesterol, triglyceride and glucose concentrations ($p<0.05$). The exposure of chickens to high temperatures causes a decrease in blood hematocrit values (Vo *et al*., 1978). This result is similar with the one by Puvadolpirod and Thaxton (2000) that reported blood corticosteron and ACTH level changes in blood cellular and chemical composition and increases in plasma concentrations of glucose, protein, triglyceride, cholesterol. Heat stress causes increase lipid peroxidation of cell membrane and dehydration of broiler chickens body and these lead to increasing MDA and hematocrit level of plasma. High environmental temperature reduced feed intake of broiler chicken (Table 2) and broilers compensate their need to energy by lipolysing of body lipid that it causes increasing the cholesterol and triglyceride of plasma. Use of diets had significant effect on cholesterol, triglycerides, MDA and glucose of serum but it had not significant effect on blood hematocrit ($p>0.05$). Compare of fish oil and tallow diet showed that

using fish oil lead to lower cholesterol, triglycerides and glucose of serum but higher MDA. Hence, additional PUFA increased cell wall lipids sensitivity to peroxidation which leads to increase serum MDA. On the other and addition of antioxidant to fish oil diet (diet A) ameliorated the negative effects and decreased lipolysis and peroxide production. Serum MDA was significantly influenced by fat type and temperature ($p>0.05$). The results showed that MDA of serum was higher in OH treatment. The probable reason for it was high temperature and high PUFA level in the ration resulted in high level of lipid peroxidation in the broilers body. This result is similar with that of Meiri *et al*. (1991).

CONCLUSION

In conclusion, heat stress decreased humoral and cell-mediate immune response and diet supplementation by vitamin E and zinc decreased the impact of heat stress on performance and immune system and also heat stress increased serum cholesterol, triglyceride, glucose and MDA and blood hematocrit.

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